Application No. 10/501,110
Amendment and Response to
Non-Final Office Action

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 3, 5, 12, 14, 15, 17 and cancel claims 2, 4, and 13 without prejudice or disclaimer. The following listing of claims will replace all prior versions, and listings, of claims in the application.

- 1. (Currently amended) An immunoassay for detecting the presence of a water-sparingly-soluble/hardly extractable protein in a sample, comprising the steps of:
- (1) extracting and/or solubilizing a water-sparingly-soluble/hardly extractable protein in a sample with an aqueous solvent containing an ionic surfactant to provide a protein solution, wherein the ionic surfactant is selected from the group consisting of sodium dodecyl sulfate, lithium dodecyl sulfate, sodium lauryl sarcosine, hexadecyltrimethyl ammonium bromide, hexadecyltrimethyl ammonium chloride, hexadecyl pyridinium chloride, and a mixture thereof and wherein the concentration of the ionic surfactant in the aqueous solvent is higher than 0.3% (W/V).
- (2) providing an immunogen for raising an antibody against the water-sparingly-soluble/hardly extractable protein to be detected, wherein the immunogen is prepared by dissolving said water-sparingly-soluble/hardly extractable protein in an aqueous solvent containing the same ionic surfactant as that contained in the aqueous solvent of step (1),
- (3) preparing the antibody against the water-sparingly-soluble/hardly extractable protein to be detected by immunizing an animal with the immunogen provided in step (2) and obtaining the antibody from the immunized animal.
- [[(2)]] (4) adding [[an-]] the antibody of step (3), wherein the antibody is raised against the water-sparingly-soluble/hardly extractable protein that is denatured previously with the ionic surfactant used in step (1), to: to the protein solution of step (1) or a dilution of the protein solution of step (1) to form a reaction mixture wherein the reaction mixture contains more than 0.03% (W/V) of the ionic surfactant contained in the aqueous solvent of step (1),
- a) the protein solution obtained in the step (1) above without substantially diluting the solution, or

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b) a dilution of the protein solution obtained in the step (1) above, wherein the protein solution is diluted such that the concentration of the ionic surfactant is not reduced to 0.03% (W/V) or less,

thereby forming an antigen antibody complex between the water-sparingly-soluble/hardly extractable protein and the antibody, and

(5) incubating the reaction mixture of step (4) to form an antigen-antibody complex between the water-sparingly-soluble/hardly extractable protein and the antibody in the presence of more than 0.03% (W/V) of the ionic surfactant contained in the aqueous solvent of step (1), and

[[(3)]] <u>(6)</u> detecting the formed antigen-antibody complex.

2. (Canceled)

3. (Currently amended) The assay according to claim 1 or 2, wherein the formation of the antigen-antibody complex in step [[(3)]] (5) is carried out in the presence of the ionic surfactant at a concentration of higher than 0.3% (W/V).

4. (Canceled)

- 5. (Currently amended) The assay according to claim [[4]] 1, wherein the ionic surfactant is sodium dodecyl sulfate.
- 6. (Previously presented) The assay according to claim 1, wherein the aqueous solvent in step (1) further comprises a reducing agent.
- 7. (Original) The assay according to claim 6, wherein the reducing agent is 2-mercaptoethanol, dithiothreitol or a mixture thereof.
- 8. (Original) The assay according to claim 7, wherein the aqueous solvent in step (1) comprises 1% (W/V) sodium dodecyl sulfate and 1M 2-mercaptoethanol.

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- 9. (Previously presented) The assay according to claim 1, wherein in step (1), the protein solution is further boiled.
- 10. (Original) The assay according to claim 9, wherein the boiling is continued at least at 80°C for 5 minutes.
- 11. (Previously presented) The assay according to claim 1, wherein the protein is selected from the group consisting of ovalbumin, ovomucoid, casein, β -lactoglobulin, buckwheat protein, wheat protein and peanut protein which are in a hardly extractable state.
- 12. (Currently amended) An antibody suitable-for use in an immunoassay for detecting the presence of a water-sparingly-soluble/hardly extractable protein in a sample according to claim 1, detecting the presence of a protein in an aqueous solvent containing an ionic surfactant, wherein the antibody is prepared by immunizing an animal against an immunogen and obtaining the antibody from the immunized animal; wherein said immunogen is prepared by dissolving the water-sparingly-soluble/hardly extractable protein to be detected in an aqueous solvent containing an ionic surfactant selected from the group consisting of sodium dodecyl sulfate, lithium dodecyl sulfate, sodium lauryl sarcosine, hexadecyltrimethyl ammonium bromide, hexadecyltrimethyl ammonium chloride, hexadecyl pyridinium chloride, and a mixture thereof; and wherein the water-sparingly-soluble/hardly extractable protein is selected from the group consisting of ovalbumin, ovomucoid, casein, β-lactoglobulin, buckwheat protein, wheat protein and peanut protein and said antibody is raised against said protein denatured with said ionic surfactant.
- 13. (Canceled)
- 14. (Currently amended) The antibody according to claim [[43]] 12, wherein the ionic surfactant is sodium dodecyl sulfate.

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15. (Currently amended) The antibody according to any one-of claims 12 to 14 claim 12 or 14, wherein the protein is denatured with the ionic surfactant under the presence of aqueous solvent further contains a reducing agent.

- 16. (Previously presented) The antibody according to claim 15, wherein the reducing agent is 2-meraptoethanol, dithiothreitol or a mixture thereof.
- 17. (Currently amended) An immunoassay kit for <u>use in an immunoassay according to claim 1, wherein the kit comprises the antibody of claim 12 and wherein the kit is for detecting the presence of a protein selected from the group consisting of ovalbumin, ovomucoid, casein, β-lactoglobulin, buckwheat protein, wheat protein and peanut protein which comprises the antibody according to any one of claims 12.</u>